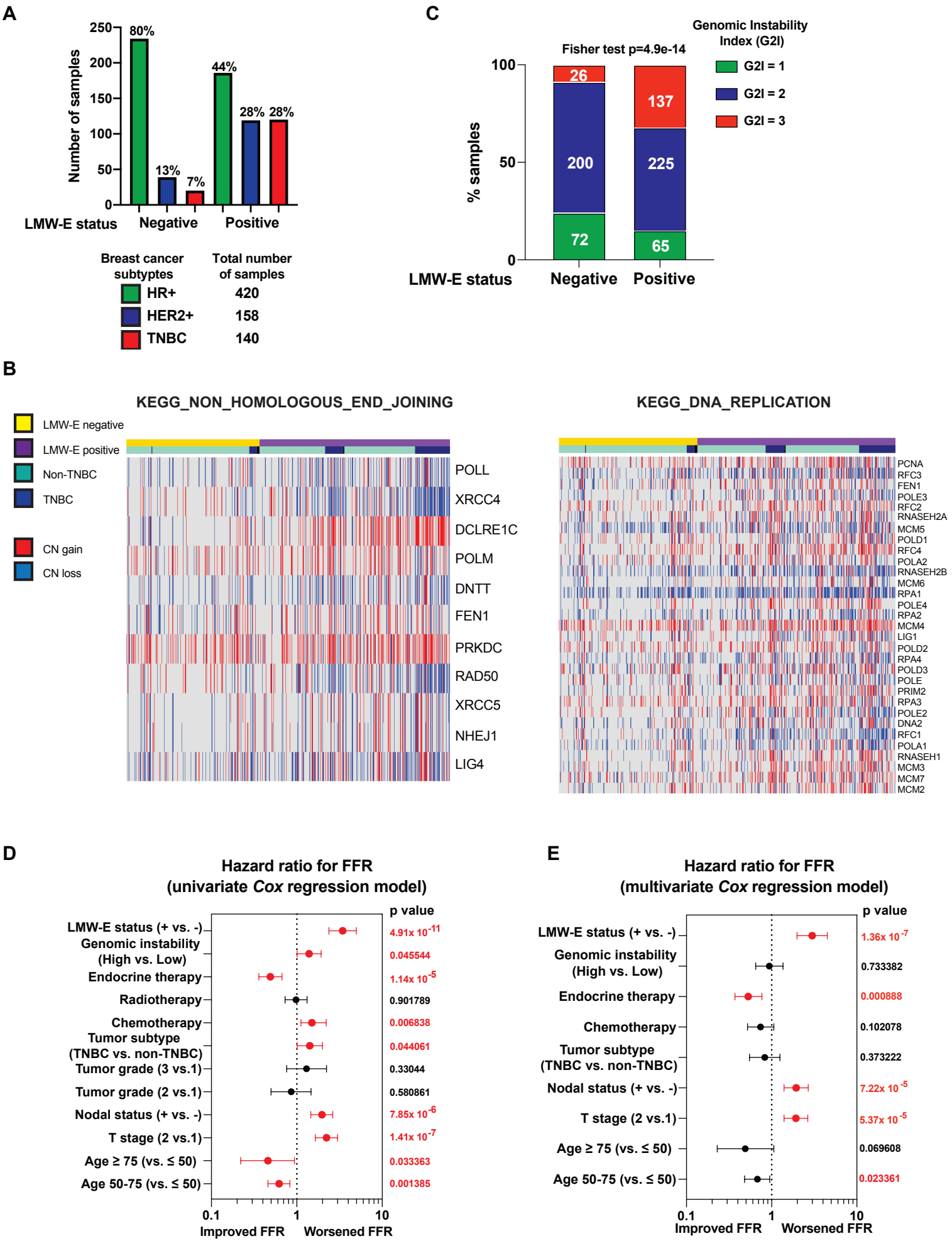
Supplementary Figure 1



Supplementary Figure 1.

A. The distribution of breast cancer subtypes stratified according to LMW-E negative and LMW-E positive patient cohorts. **B.** Heatmaps showing the copy number (CN) gains (red) and losses (blue) of genes in KEGG non-homologous end jointing pathway (left panel) and KEGG DNA replication pathway (right panel) in LMW-E positive sample group compared with LMW-E negative sample group. TNBC status is also indicated in the horizontal bar graphs on top of the heat maps. **C.** The distribution of tumor samples with stable genomes (G2I-1; n = 137), intermediate stable genomes (G2I-2; n= 425), and unstable genomes (G2I-3; n= 163) in LMW-E negative and LMW-E positive patient cohorts. **D.** Univariate *Cox* regression analyses associated with improved or worsened freedom from recurrence (FFR) in the cohort of 725 stage I and II breast cancer patients. Hazard ratio and 95% confidence interval from univariate *Cox* regression model are shown in the forest plot, and the p-value for each of the analyzed variables are listed on the right and highlighted in red if $p < 0.05$. **E.** Multivariate *Cox* regression analyses for the factors significantly associated with improved or worsened freedom FFR (based on univariate *Cox* regression in panel D). Hazard ratio and 95% confidence interval from multivariate *Cox* regression model are shown in the forest plot, and the p-values for each of the variables are listed on the right and highlighted in red if $p < 0.05$.

Supplementary Figure 2

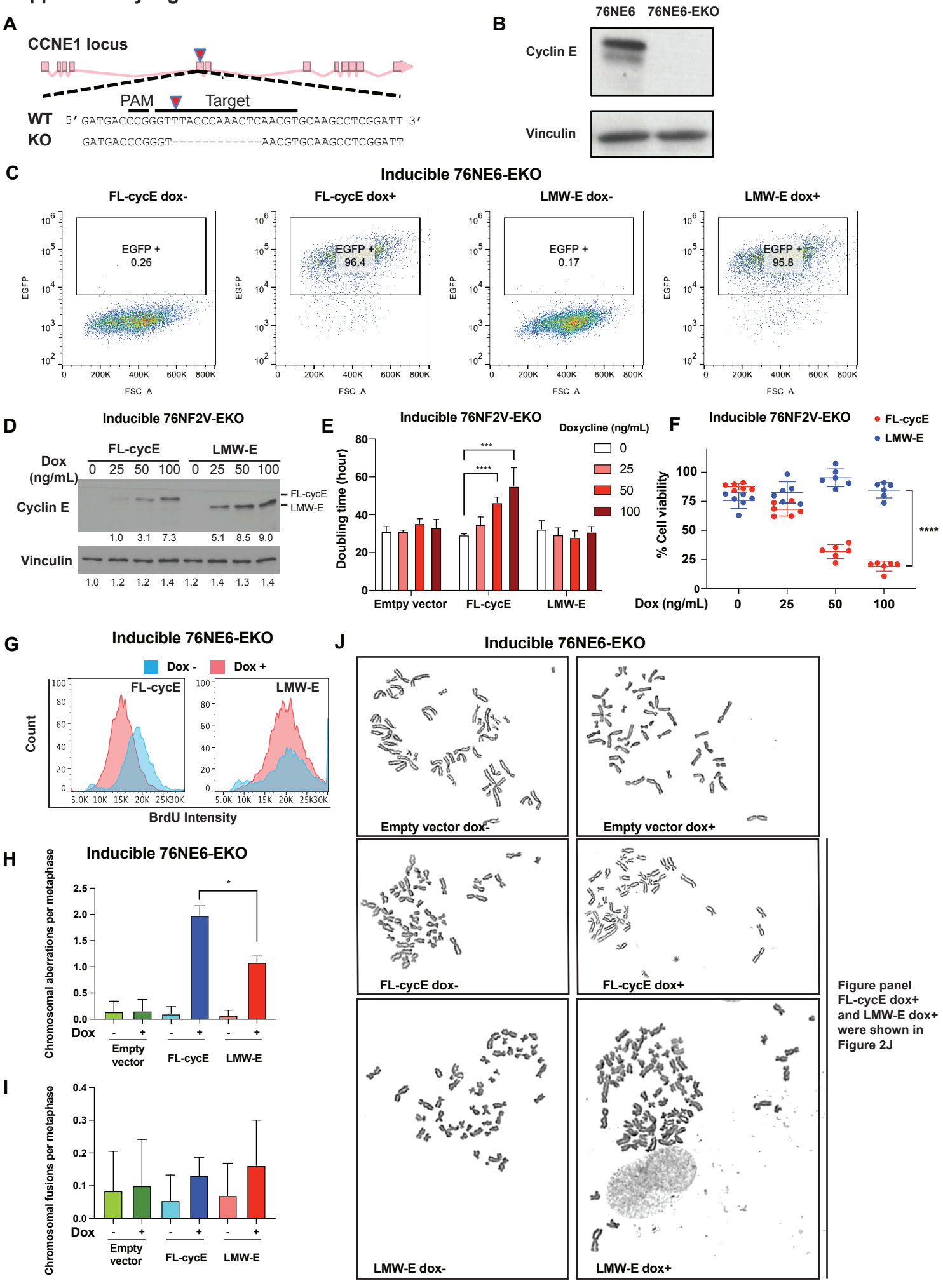
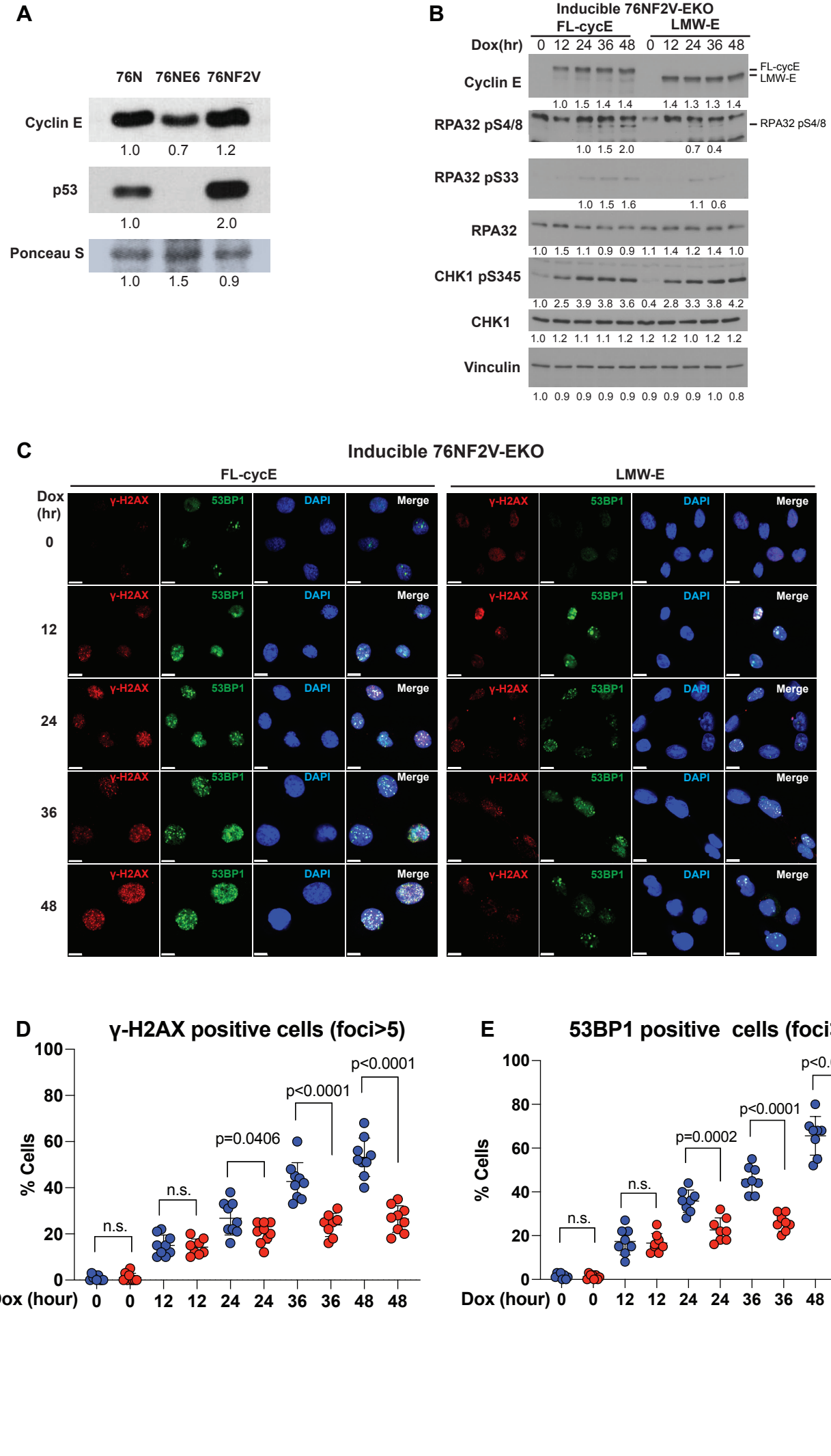


Figure panel FL-cycE dox+ and LMW-E dox+ were shown in Figure 2J

Supplementary Figure 2.

A and B. generation of *CCNE1* knock-out hMEC cell line by CRISPR/gRNA. The CRISPR deletion of gRNA target in *CCNE1* locus is confirmed by Sanger sequencing (A), and the depletion of endogenous cyclin E protein expression was confirmed by western blot analysis (B). **C.** FACS analysis for the ratio of EGFP (fused to c-terminus of inducible FL-cycE or LMW-E and expressed when FL-cycE or LMW-E were induced by doxycycline) positive inducible 76NE6-EKO cells with or without 24 hours treatment of 100ng/mL doxycycline. **D.** LMW-E and FL-cycE protein expression were examined by western blot analysis in the inducible 76NF2V- EKO cell lines after 36 hours treatment of doxycycline at the indicated concentrations. **E.** Cell doubling times were monitored by cell confluency mask from live cell imaging (Incucyte) in inducible 76NF2V-EKO cells after induced expression of LMW-E, FL-cycE or empty vector by doxycycline treatment at the indicated concentrations (n=4, mean with standard deviation). **F.** Cell viability calculated by MTT assay after induced expression of LMW-E or FL-cycE in inducible 76NF2V-EKO by treatment with doxycycline at the indicated concentrations, normalized by empty vector control (n=4, mean with standard deviation). **G.** DNA synthesis in inducible 76NE6- EKO cell lines with or without LMW-E or FL-cycE over-expression were assessed by bromodeoxyuridine (BrdU) incorporation and analyzed by FACS. **H - I.** 76NE6-EKO cells following 36 hours of induced expression of LMW-E or FL-cycE were subjected to metaphase spread assay to assess the extent of chromosomal instability-results show enumerations of total chromosomal aberration frequency (H) and chromosomal fusion frequency (I). Two biological repeats, each with 35 metaphase preparations for each condition. **J.** Representative metaphase spread images for inducible 76NE6-EKO empty vector, FL-cycE and LMW-E cells. Note that the representative images for FL-cycE dox+ and LMW-E dox+ were also shown in main Figure 2J. ***P<0.001 and ****P<0.0001, Student *t* test.

Supplementary Figure 3

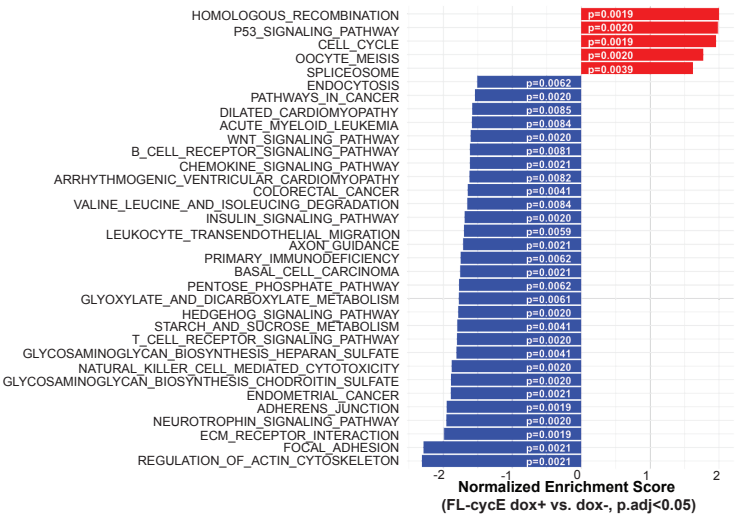


Supplementary Figure 3.

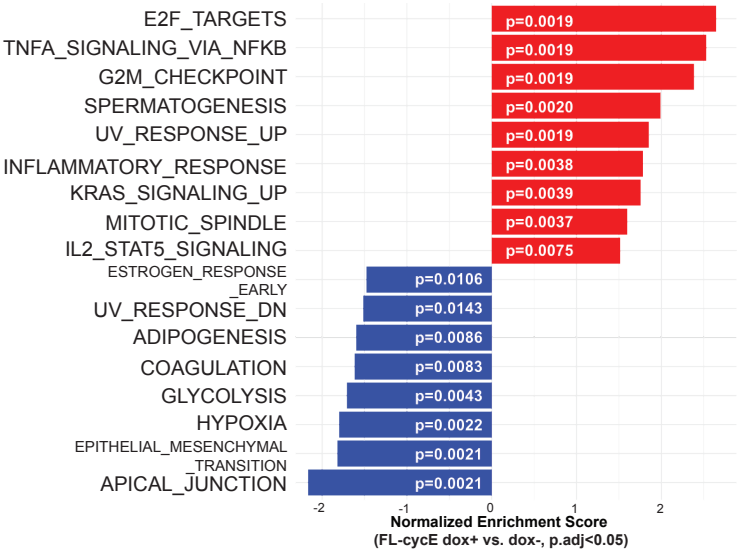
A. Western blot analysis of cyclin E and p53 in 76N (mortal cell strains), 76NE6 and 76NF2V cell lines. **B.** Western blot analysis of the indicated replication stress markers in 76NF2V-EKO cells following induced expression of LMW-E or FL-cycE. Cells were treated with 100ng/ml doxycycline in a time course dependent manner. Uninduced control (Dox 0 hour) were treated with DMSO for 48 hours. **C.** Representative images of immunofluorescent analysis of γ-H2AX and 53BP1 foci in 76NF2V-EKO cells following induced expression of LMW-E or FL-cycE (scale bar=10 μm). **D and E.** Quantitation of time course analysis of γ -H2AX (panel D) and 53BP1(panel E) positive cells (nuclear foci>5) in 76NF2V-EKO cell lines induced for FL-cycE or LMW-E expression (100ng/mL doxycycline for induction, DMSO for uninduced control, cell number>600, Mean with standard deviation, p values shown in graph, n.s.: not significant, Student *t* test).

Supplementary Figure 4

A KEGG pathways significantly enriched by FL-cycE overexpression



B HALLMARK pathways significantly enriched by FL-cycE overexpression



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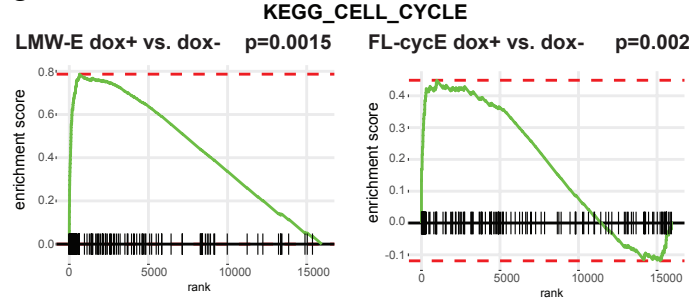
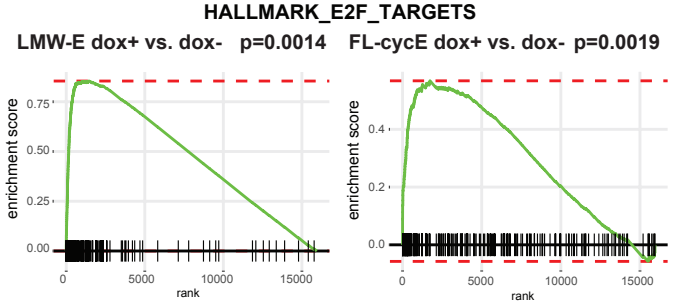
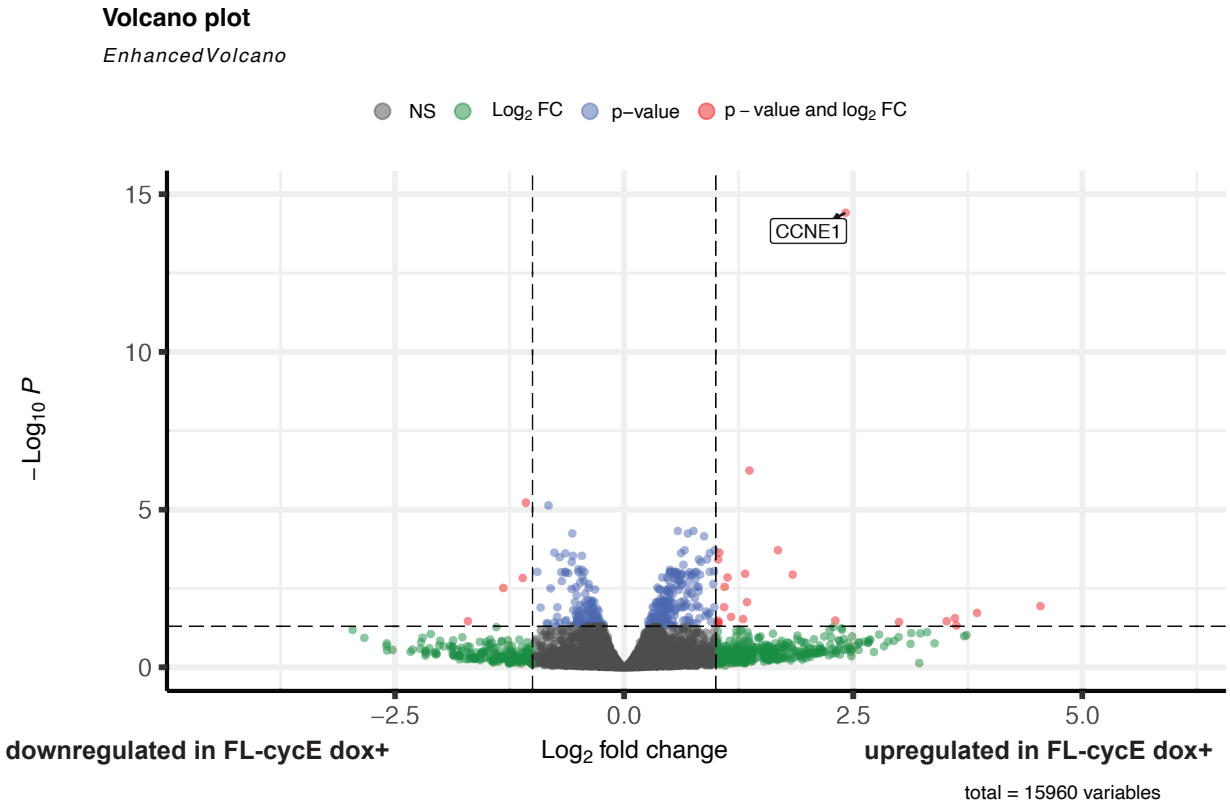


D



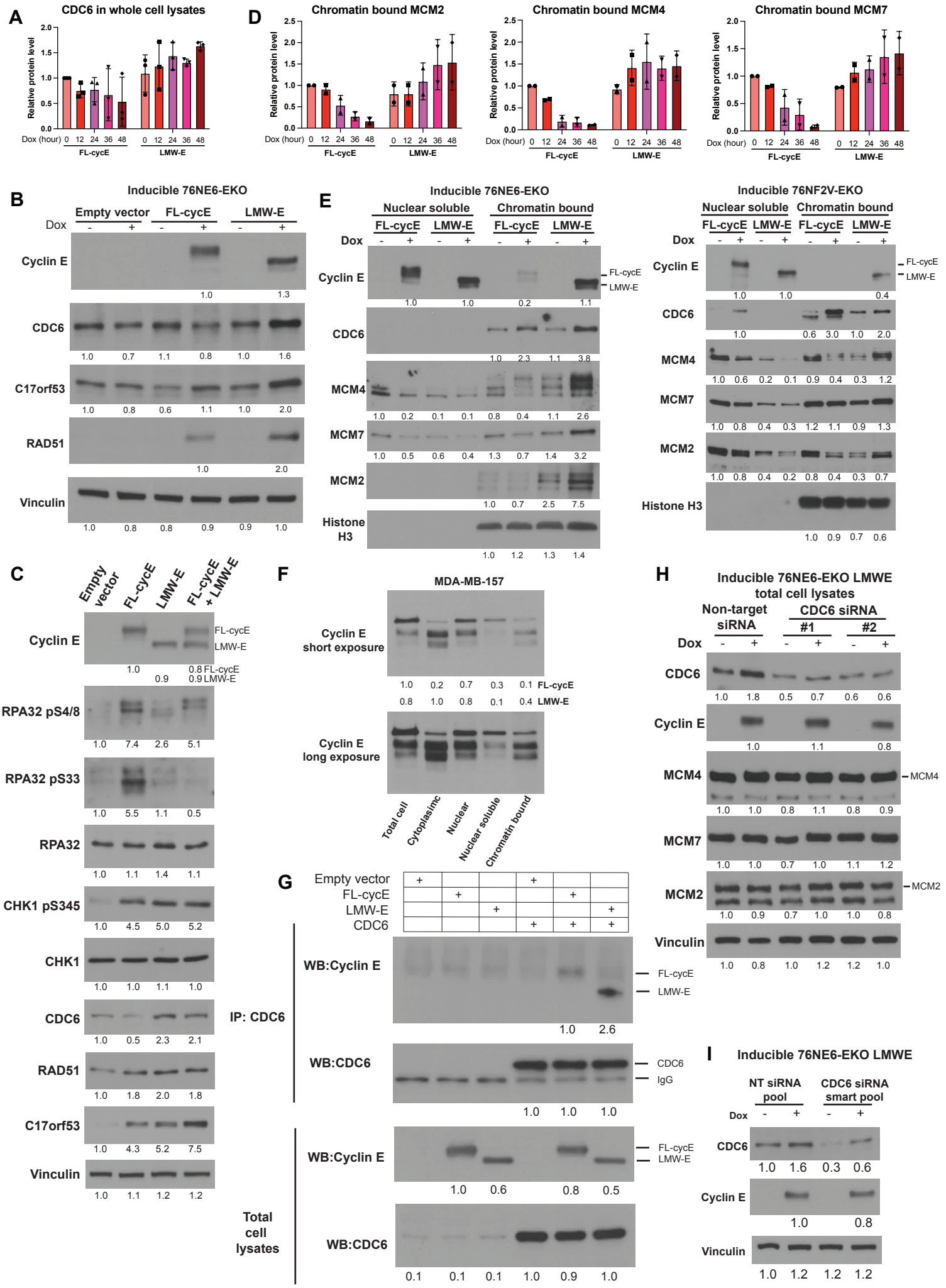
E FL-cycE dox+ vs. dox-



Supplementary Figure 4.

A. Waterfall plots of the KEGG pathways significantly enriched in inducible 76NE6-EKO FL-cycE cells treated with 100ng/ml doxycycline for 36 hours, followed by RNA-seq and transcriptional profiling analysis. Inducible 76NE6-EKO FL-cycE cells cultured without doxycycline (dox-, DMSO added) were served as reference (adjusted $p<0.05$). **B.** Waterfall plots of HALLMARK pathways significantly enriched in inducible 76NE6-EKO FL-cycE dox+ group, compared to FL-cycE dox- group (adjusted $p <0.05$). **C.** Enrichment plots of KEGG cell cycle gene sets in LMW-E dox+ group compared to LMW-E dox- group (left panel) and FL-cycE dox+ group compared to FL-cycE dox- group (right panel). **D** Enrichment plots of HALLMARK E2F targets gene set in LMW-E dox+ group compared to LMW-E dox- group (left panel) and FL-cycE dox+ group compared to FL-cycE dox- group (right panel). **E** Volcano plot to compare the expression profiles between FL-cycE dox+ and FL-cycE dox- 76NE6-EKO cell lines. *CCNE1* is the top ranked gene overexpressed in FL-cycE dox+ cells.

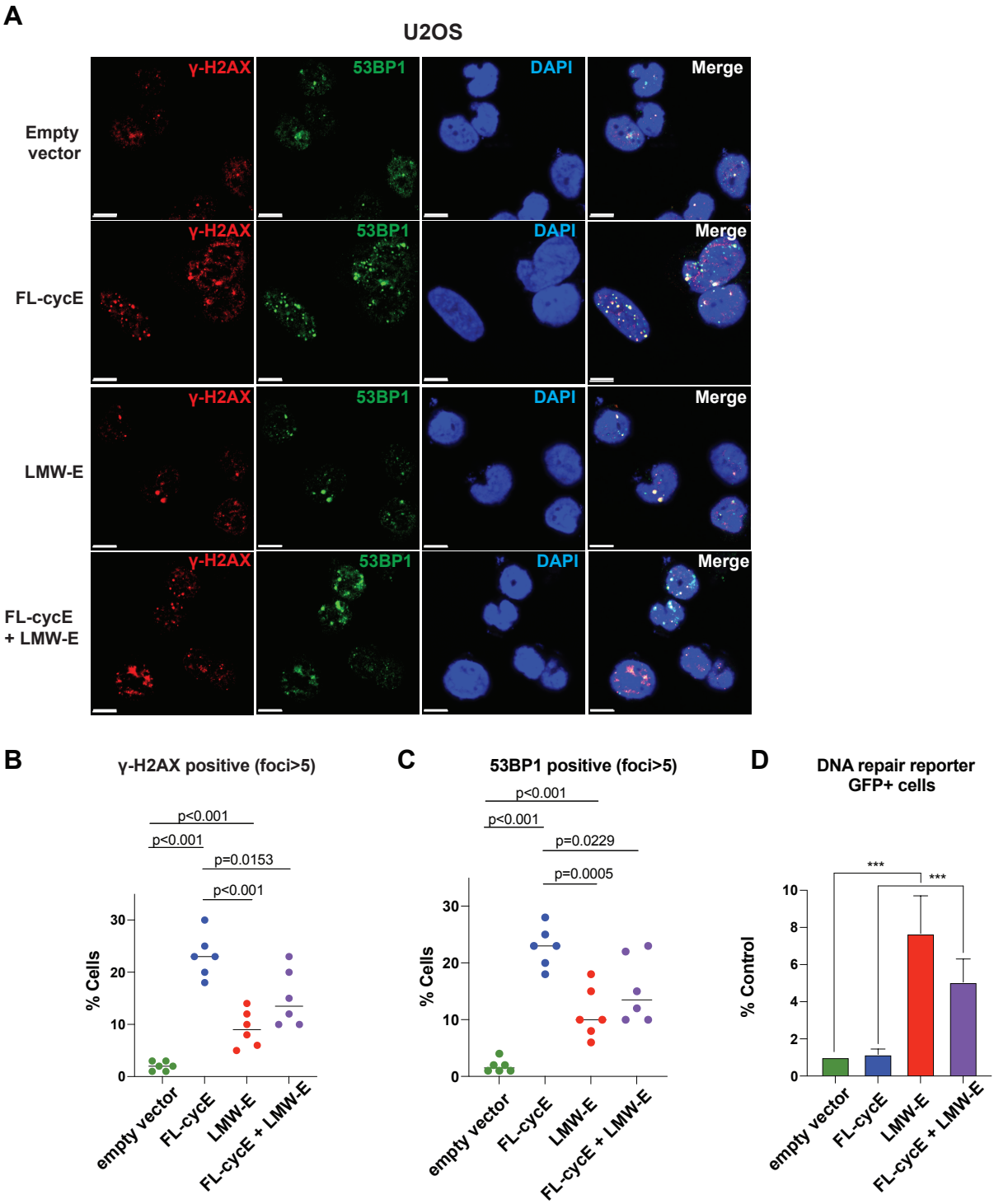
Supplementary Figure 5



Supplementary Figure 5.

A. Densitometry and quantitation of CDC6 protein levels (*see* Figure 5B) in whole cell lysates collected from inducible 76NE6-EKO cells with time-course expression (0-48 hours) of FL-cycE or LMW-E (3 biological repeats). **B.** Western blot analysis of cyclin E, CDC6, C17orf53, and RAD51 in inducible 76NE6-EKO cell lines cultured with or without doxycycline (100 ng/mL, 24 hours) to induce the expression of LMW-E, FL-cycE, or empty vector control. **C.** Western blot analysis of the effect of LMW-E and FL-cycE on the indicated replication stress markers phospho-CHK1 and phospho-RPA32 in U2OS cells. The levels of CDC6, RAD51 and C17orf53 were also examined by western blot analysis in the same experiment. **D.** Densitometry and quantitation of of MCM2, MCM4 and MCM7 protein levels (see Figure 5C) in chromatin bound fractions collected from inducible 76NE6-EKO cells with time-course expression (0-48 hours) of FL-cycE or LMW-E (2 biological repeats). **E.** Western blot analysis of cyclin E (FL-cycE or LMW-E) and the indicated DNA pre-replication complex proteins in the inducible 76NE6-EKO cells (left panel) and 76NF2V-EKO cells (right panel) with or without treatment with doxycycline to induce LMW- E or FL-cycE expression. Western blot analysis was performed using protein lysates from cell nuclear soluble fraction and non-soluble (chromatin bound) fraction. **F.** Western blot analysis of FL-cycE or LMW-E in MDA-MB-157 in total cell lysates as well as the indicated fractionated lysates. **G.** Immunoprecipitation (IP) followed by western blot analysis to show the binding between cyclin E (FL-cycE or LMW-E) and CDC6 in HEK293T cells transfected with the indicated plasmids. Top panel shows the IP/westerns blot analysis and bottom panels show the input by western blot analysis. **H and I.** Western blot analysis of CDC6 and indicated proteins in inducible 76NE6- EKO LMW-E cells transfected with two individual siRNAs (H) and siRNA smart pool (I) targeting CDC6 or non-target siRNA controls. These cells were transfected with indicated siRNAs, followed by 24 hours treatment of 100ng/mL doxycycline to induce LMW-E expression.

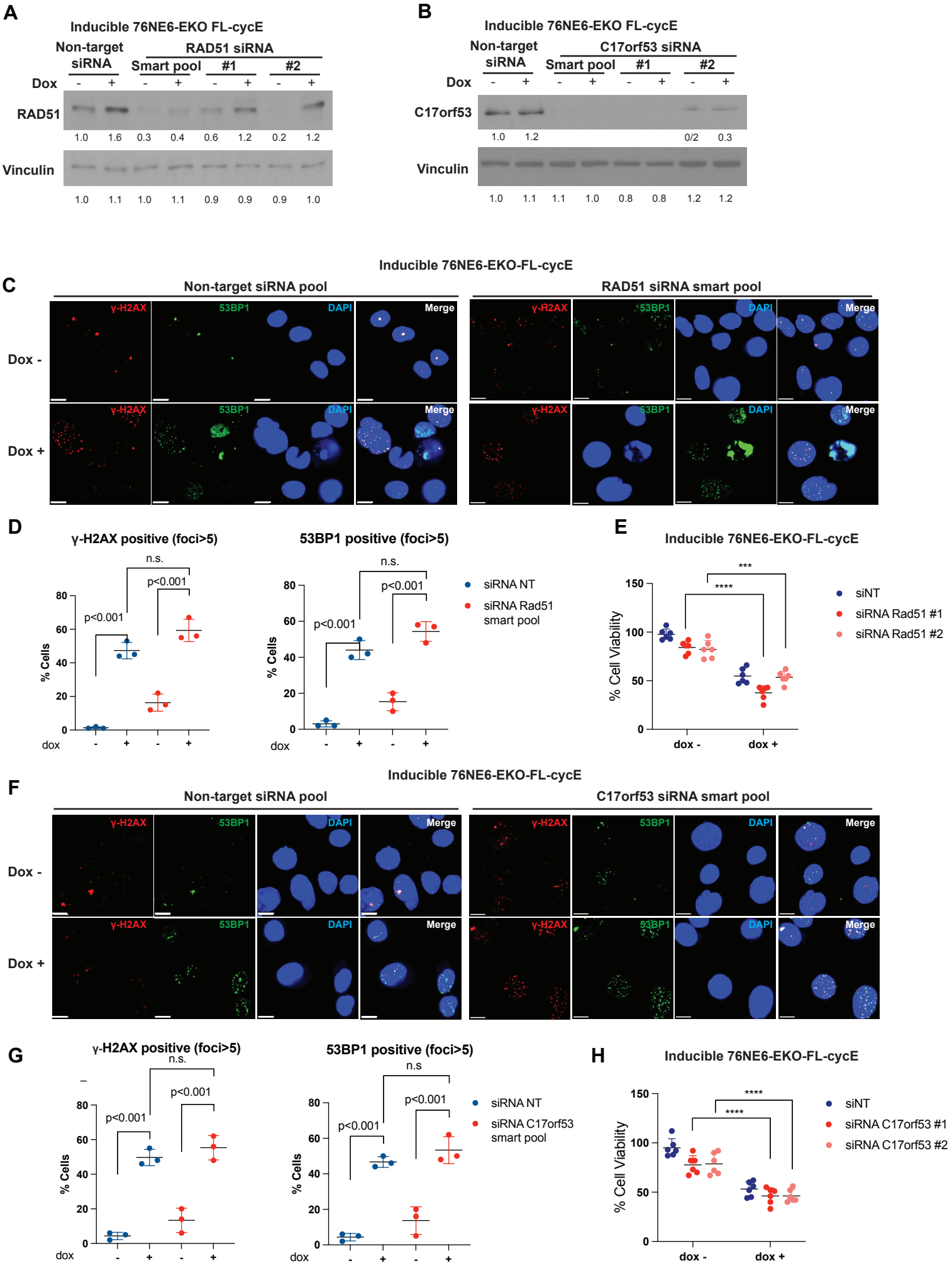
Supplementary Figure 6



Supplementary Figure 6.

A - C. Assessment of the effect of LMW-E and FL-cycE on DNA damage intensity by IF for γ-H2AX and 53BP1 foci. Representative immunofluorescence images are shown in panel A and quantification for positive cells (foci>5) are illustrated in dot-plots (panel B and C; cell number>300, mean with standard deviation, scale bar=10 μm). **D.** EJ5-GFP DNA repair reporter assay. U2OS cells engineered to express EJ5-GFP DNA repair reporter and pCBASce plasmid (an I- SceI expression vector to induce DNA double strand break), were transfected with empty vector, FL-cycE, LMW-E or FL- cycE + LMW-E followed by FACS analysis to detect and quantitate for GFP positive cells. Values are normalized with the control group. Error bars represent mean standard deviation (n=3 independent experiments, ***P<0.001) **E.** Western blot analysis of RAD51 knock-down efficiency in inducible 76NE6-EKO LMWE cells transfected with or without siRNA smart pool targeting RAD51 in cells cultured in the presence or absence of doxycycline. **F.** Western blot analysis of C17orf53 knock-down efficiency in inducible 76NE6-EKO LMWE cells transfected with or without siRNA smart pool targeting C17orf53 in cells cultured in the presence or absence of doxycycline.

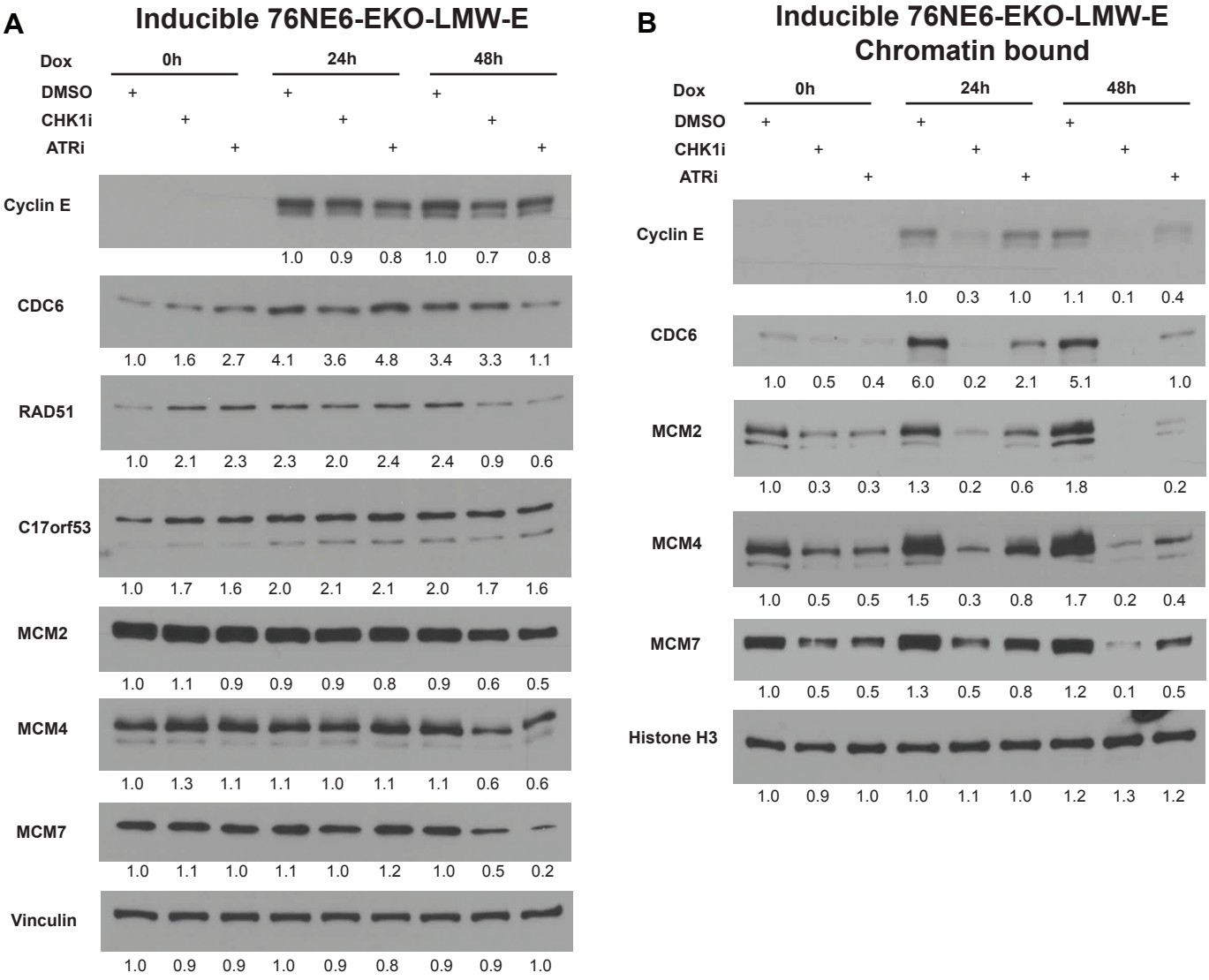
Supplementary Figure 7



Supplementary Figure 7.

A. Western blot analysis of RAD51 knock-down efficiency in inducible 76NE6-EKO FL-cycE cells. The cells were treated with 100 ng/mL doxycycline (or DMSO control) for 24 hours after indicated siRNA transfection to induce FL-cycE expression. Non-target (NT) siRNA was used as a control. **B.** Western blot analysis of C17orf53 knock-down efficiency in inducible 76NE6-EKO-FL-cycE cells. The cells were treated with 100 ng/mL doxycycline (or DMSO control) for 24 hours after siRNA transfection. Non-target (NT) siRNA was used as a control. **C.** Analysis of DNA damage intensity by immunofluorescence assay for γ-H2AX and 53BP1 foci in inducible 76NE6-EKO-FL-cycE cells (scale bar=10 μm). Cells were treated with siRNA targeting RAD51, followed by 24 hours of treatment with 100 ng/mL doxycycline to induce FL-cycE expression. Non-target siRNA and DMSO were used as controls. **D.** Quantification of γ-H2AX and 53BP1 foci in panel C (cell number > 150, mean with standard deviation). **E.** Analysis of cell viability by MTT assay in inducible 76NE6-EKO-FL-cycE cells after transfection with specific siRNAs targeting RAD51, followed by treatment with 100 ng/mL doxycycline to induce FL-cycE expression for 48 hours Non-target siRNA and DMSO (dox-) were used as controls. **F**. Analysis of DNA damage intensity by immunofluorescence assay for γ-H2AX and 53BP1 foci in inducible 76NE6-EKO-FL-cycE cells (scale bar=10 μm). Cells were treated with siRNA targeting C17orf53, followed by 24h hours of treatment with 100 ng/mL doxycycline to induce FL-cycE expression. Non-target siRNA and DMSO were used as controls. **G.** Quantification of γ-H2AX and 53BP1 foci in panel **F** (cell number > 150, mean with standard deviation). **H.** Analysis of cell viability by MTT assay in inducible 76NE6-EKO cells after transfection with specific siRNAs targeting C17orf53, followed by 100 ng/mL doxycycline to induce FL-cycE expression for 48 hours. Nontarget siRNA and DMSO (dox-) were used as controls. For all statistical analyses, ***$p < 0.001$ and ****$p < 0.0001$, Student *t* test.

Supplementary Figure 8



Supplementary Figure 8.

A. Western blot analysis for the levels of LMW-E, CDC6, C17orf53, RAD51, MCM2, MCM4 and MCM7, using whole cell lysates collected from inducible 76NE6-EKO-LMW-E cells with CHK1 inhibitor rabusertib (70nM), or ATR inhibitor ceralasertib (125nM; both at their respective IC50 concentrations), with or without LMW-E expressing for 24 and 48 hours. **B.** Western blot analysis for chromatin bound LMW-E, CDC6, MCM2, MCM4 and MCM7 in inducible 76NE6-EKO-LMW-E cells treated using the same strategy as panel (A).